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Serial No. : 09/676,526 Examiner : Mutschler, Brian
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Title : ELECTROPHORETIC ANALYSIS SYSTEM HAVING IN-SITU CALIBRATION

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1. (Withdrawn) A parallel electrophoresis system having a plurality of separation lanes, a detector and a processor connected to the detector, wherein light intensity is received at the detector from at least two different separation lanes, and processed using at least two different calibration matrices.

2. (Currently Amended) A method of calibrating a detection system in an electrophoresis apparatus comprising ~~of~~ at least one separation lane, the detection system configured to sense a spectrum of light intensities over a number m wavelength channels from said at least one separation lane, the method comprising:

detecting at least one spectrum of light intensities for each of a plurality of samples;

clustering the detected spectra of light intensities into a number n categories by using at least one specified clustering criterion ~~criteria~~, the step of clustering comprising:

normalizing light intensities of each of the detected spectra by a respective normalization value to prepare spectra comprising normalized light intensities;

comparing corresponding normalized light intensities of different spectra; and

clustering detected spectra that do not have compared corresponding normalized light intensities differing by more than the specified clustering criterion; and

creating a calibration matrix from the clusters.

3. (Original) The method according to claim 2, wherein detected spectra of at least some samples are discarded prior to the clustering step.

4. (Original) The method according to claim 2, wherein a calibration matrix is determined for each of a corresponding plurality of separation lanes.

5. (Original) The method according to claim 4, wherein a total of at least 96 calibration matrices are generated, one for each of a corresponding separation lane.

6. (Withdrawn) An electrophoresis separation apparatus having at least one separation lane, a detector, and a processor, wherein the apparatus is configured to:

detect at least one spectrum of light intensities for each of a plurality of samples;

a cluster the detected sets of light intensities into a number *n* categories by using specified clustering criteria; and

create a calibration matrix from the cluster.

7. (Withdrawn) The electrophoresis separation apparatus according to claim 6, wherein the apparatus is configured to discard the spectra of at least some samples prior to the clustering step.

8. (Withdrawn) A method of identifying nucleotides in an electrophoretically separated DNA sample which has been tagged with a chromophore, comprising:

displaying light intensities on a two dimensional time-wavelength plot; and

identifying nucleotides based upon the shape and position of said formations displayed on said plot.

9. (Withdrawn) An electrophoretic detection system for separating a sample containing therein a plurality of dye components, wherein the detection apparatus is configured to automatically determine the number of different dye components from spectra of the sample.

10. (Currently Amended) A method for automatically calibrating an electrophoretic separation apparatus having a plurality of separation lanes, the method comprising the steps of:

for each separation lane, detecting a plurality of sets of light intensities from a migrating sample, the light intensities in each set being collected in a total of R channels, where $R \geq 2$;

for each separation lane, ~~isolating~~ identifying isolated peaks in at least some of the plurality of sets of light intensities;

estimating a number of dyes M present in the migrating sample based on the isolated peaks, where $M \geq 2$; ~~and~~

for each separation lane, calculating coefficients based on the distribution of light intensities in ~~the~~ channels corresponding to ~~of~~ the isolated peaks, wherein the coefficients map detected light intensities from the R channels onto values reflective of the relative likelihood of each of the dyes being present;

wherein estimating a number of dyes comprises:

normalizing light intensities of each of the respective sets of light intensities with respect to a respective normalization value;

comparing normalized light intensities of different sets of light intensities;

clustering detected sets of light intensities that do not have compared corresponding normalized light intensities differing by more than a specified clustering criterion; and

preparing a calibration matrix using the clustered sets of light intensities.

11. (Original) A method according to claim 10, wherein the coefficients are arranged in the form of an R x M matrix.

12. (Cancelled) A parallel electrophoresis system having a plurality of separation lanes for simultaneous separation of a sample in each of the separation lanes, a detector and a processor connected to the detector, wherein the processor is configured to simultaneously process light detected from species tagged with dyes belonging to more than one dye set.

13. (Currently Amended) A method for automatically calibrating a separation apparatus, said method comprising the steps of:

sampling light emitted from species having a chromophore, the sampling being performed over a first number *m* of wavelength channels and a second number *n* of time intervals to thereby form a time-wavelength distribution wherein a total of *k* discrete species are represented by morphological formations in the ~~said~~ time-wavelength distribution;

isolating a total of *l* peaks from said formations, each peak corresponding to a discrete species;

clustering the *l* peaks into a number *j* classes based on at least one similarity criterion, wherein clustering the *l* peaks comprises:

normalizing wavelength values of the time-wavelength distribution; and

identifying peaks that do not have corresponding normalized wavelength values differing by more than the at least one similarity criterion;

forming a total of *j* calibration vectors, each calibration vector representing one of said classes; and

forming a calibration matrix A comprising ~~of~~ the j calibration vectors.

14. (Currently Amended) The method of claim 13, wherein ~~the method of~~ isolating *l* peaks from the formations comprises ~~of~~:

preprocessing the sample data in the time domain;

isolating a total of *p* peaks in the time domain; and

isolating a total *l* peaks from *p* peaks according to the width and spacing of said peaks in the time domain[;].

15. (Original) The method of claim 13, wherein the step of isolating peaks comprises employing morphological filters to identify peaks in the time-wavelength distribution.

16. (Original) The method of claim 13, wherein the step of isolating peaks comprises visually inspecting the time-wavelength distribution and selecting those peaks which are unconnected to other peaks.

17. (New) A method for calibrating an electrophoresis system, comprising:

electrophoretically separating a sample along a separation lane;

obtaining, at a number t different times, a spectrum indicative of the presence of sample components separated along the separation lane, each spectrum comprising a number x intensity values, the x intensity values of each spectrum being indicative of an intensity at a different wavelength;

normalizing a plurality of the x intensity values of each of a plurality of the t spectra to a respective normalization value;

clustering spectra having normalized intensity values by steps comprising:

(a) comparing normalized intensity values of a first one of the spectra with corresponding normalized intensity values of another spectrum;

(b) assigning the other spectrum to a first cluster including the first spectrum if a maximum difference between the compared normalized fluorescence intensity values of the first spectrum and the other spectrum is less than a specified value;

(c) repeating the comparing and assigning steps until normalized fluorescence intensity values of all of the other spectra have been compared with corresponding normalized fluorescence intensity values of the first one of the spectra;

(d) comparing normalized fluorescence intensity values of a second spectrum, the second spectrum not assigned to a cluster, with corresponding normalized fluorescence intensity values of another spectrum not assigned to a cluster;

(e) assigning the other spectrum of step (d) to a new cluster including the second spectrum if a maximum difference between the compared normalized fluorescence intensity values of the second spectrum and the other spectrum of step (d) is less than a specified value;

(f) repeating the (d) comparing and (e) assigning steps until all of the other spectra not yet assigned to a cluster have been compared with the second spectrum; and

(g) repeating the (d) comparing, (e) assigning, and (f) repeating steps until all of the spectra have been assigned to a respective cluster.

18. (New) A method for processing separations data, comprising:

electrophoretically separating a sample along a separation lane in the presence of a number n fluorescent dyes, the fluorescence of each of the n dyes being indicative of the presence of a different component of the sample, each dye having a different fluorescence spectrum;

obtaining, at a number t different times, a fluorescence spectrum indicative of the presence of one or more components of the sample separated along the separation lane, each spectrum comprising a number x intensity values, the x intensity values of each spectrum being indicative of an intensity at a respective, different wavelength;

normalizing a plurality of the x intensity values of a plurality of the t spectra to a respective normalization value;

selecting a number n subsets of the spectra with normalized x intensity values, the members of each subset determined by comparing the normalized intensity values of respective spectra with corresponding normalized intensity values of at least one of the other spectra and clustering in a subset those spectra that do not have compared corresponding normalized intensity values differing by more than a specified value.

19. (New) The method of claim 18, wherein the spectra of each one of the n subsets are indicative of the presence of one of the n dyes.

20. (New) The method of claim 19, wherein the number n subsets comprise fewer than t members and the method further comprises using the members of the n subsets to determine the presence of the n dyes in the spectra not clustered in one of the n subsets.

21. (New) The method of claim 20, wherein using the members of the n subsets comprises creating a calibration matrix from the n subsets.

22. (New) The method according to claim 21, wherein a calibration matrix is determined for each of a corresponding plurality of separation lanes.